#12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	:	122
Examiner	:	W. Teoli
Applicant(s)	:	Mahendra I. Amin and Jay A. Campbell
Serial Number	:	Continuation of 06/898,676
Filed	:	
For	:	CRYSTALLINE CEPHALOSPORIN HYDROHALIDE SALTS

Commissioner of Patents and Trademarks
Washington, DC 20231

DECLARATION UNDER 37 CFR 1.132

Sir:

I, Jay A. Campbell, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 USC 1001) and may jeopardize the validity of the above-captioned application or any patent issuing thereon, state and declare that:

All statements herein made of my own knowledge are true and all statements herein made on information and belief are believed to be true.

1. I am one of the named co-inventors in the above-identified application.

2. I am a PhD level chemical engineer having received my PhD degree from the University of Illinois in 1981. I have worked for The Upjohn Company (hereinafter, Upjohn) since 1981; I have been assigned to work in Process Research and Development for Upjohn since 1981.

3. Among my assignments are chemical laboratory studies to find more economical, safer and easier ways to handle relatively new chemical compounds of interest to Upjohn for possible large production scale manufacture and commercialization.

4. Pertinent to the hereinabove identified patent application, I was assigned the process research task of trying to find a better, easier handling form of ceftiofur, a cephalosporin antibiotic compound that Upjohn was inter-

ested in possibly marketing.

5. The compound ceftiofur itself, syn-7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylic acid and its cationic metal and amine salts are amorphous solids which amorphous materials are difficult to purify, and are typically less stable than crystalline materials, and are less desirable to work with relative to crystalline materials in the plant manufacturing thereof for pharmaceutical formulations containing such a compound.

6. My initial task or problem relating to this ceftiofur synthesis was to find a commercially adaptable process to make a pure form of ceftiofur sodium salt. No method of purification of either the ceftiofur sodium salt or the ceftiofur free base we tried resulted in acceptable purity of a final ceftiofur material. Among those methods we tried were (1) reprecipitations of the ceftiofur sodium salt, (2) resin and other adsorbent chromatographies, (3) reprecipitation of the ceftiofur free base, (4) various changes in reaction conditions, (5) extractions and leachings, and (6) purification of the protected ceftiofur.

7. We then looked for a crystalline intermediate or crystalline form of ceftiofur sodium which could be purified by crystallization. In the course of our development of the detritylation part of the overall ceftiofur compound synthesis with HCl we formed a crystalline solid, later confirmed to be ceftiofur HCl. We then set about to optimize this detritylation and crystallization.

8. The accepted, published methods of detritylating cephalosporins use formic acid or trifluoroacetic acid. Those detritylation acids are not as harsh as mineral acids. Hydrochloric acid and hydrobromic acid are known to be harsher and cause degradation of the cephalosporin. In spite of this, in our work with ceftiofur, we observed very clean detritylation in vials for high performance liquid chromatography (HPLC) injection samples which had been diluted with acetonitrile, water and HCl. This hydrochloric acid detritylation of ceftiofur has the advantages of involving better known and less hazardous reagents (in high volume plant use), easier workup and easier formation of the HCl salt. However, when we did this hydrochloric acid detritylation of ceftiofur to form the ceftiofur hydrochloride, we unexpectedly obtained our ceftiofur hydrochloride salt product as a crystalline material upon cooling of

the mixture. This material was not substantially degraded, and it was found upon analysis that the crystalline ceftiofur hydrochloride salt retained essentially all of its syn-stereo configuration.

9. We also found that this crystalline ceftiofur hydrochloride salt was of very high purity and was, in fact, a more pure form of ceftiofur than we (at Upjohn) had been able to obtain previously. In fact, the hydrochloride salt we obtained was over 10% purer than our best sample of sodium salt. As a result of this discivery, Upjohn management set about to consider and to test using the ceftiofur hydrochloride as a possible new form or source of ceftiofur as a bulk drug for formulating into appropriate pharmaceutical dosage unit forms, instead of further converting it into the previously known sodium ceftiofur salt.

10. From our work with different cephalosporin intermediates, we were surprised that our crystalline ceftiofur hydrochloride salt was so impurity-free. For example, attached hereto are copies of my laboratory notebook page 18774-JAC-45 noting my efforts to make and crystallize the hydrochloride salt of the corresponding 3-(thienylcarbonylthiomethyl) compound, namely 7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetamido]-3-[2-(2-thien-2-ylcarbonyl)thio-methyl]-3-cephem-4-carboxylic acid, by processing the corresponding free base in the same way we would process the N-tritylceftiofur compound.

11. The 3-(thienylcarbonylthiomethyl) ... free base was dissolved with dilute hydrogen chloride in about 35:65 V/V aqueous acetone, then distilled to remove acetone with concurrent crystallization of the salt. The resulting product was crystalline but over two percent of the anti-isomer was formed compared to less than one percent in the corresponding ceftiofur process. The crystals were much smaller which made the crystal isolation slower.

12. In another experiment (see copy of my laboratory notebook page 18774-JAC-47, attached hereto), we treated cefotaxime (identical to ceftiofur except for the 3-side chain) with warm aqueous acetone and hydrogen chloride. The resulting product contained less than 50 percent of the desired cefotaxime hydrochloride salt.

13. Overall, a surprising advantage of the ceftiofur hydrochloride salt is that it easily conforms to a crystalline habit. As exemplified above, few cephalosporin salts form crystals, and thus, this result is unex-

pected. Furthermore, the crystalline form of ceftiofur hydrochloride salt has the purity and handling advantages noted above. Its stable, solid crystalline form makes it easier to purify, handle, store, process and formulate and its higher purity makes it more suitable for formulating into appropriate pharmaceutical dosage unit forms.

14. To our knowledge, to date, there are no other crystalline forms of ceftiofur, including any of the specific compounds referred to in the Labeeuw et al. U.S. patent No. 4,464,367. This crystalline ceftiofur hydrochloride salt is believed to be a unique form of ceftiofur.

15. Amorphous ceftiofur hydrochloride salt has been made by recrystallization from a weakly acidic solution (see copy of notebook page 19706-KPS-114, attached hereto) and may be made by other methods known to one of ordinary skill in the art, for example, by spray-drying or freeze-drying. Both crystalline and amorphous forms would be expected to be more stable than an amorphous metal salt of ceftiofur because of their lower pKa. Freeze-dried or spray-dried ceftiofur hydrochloride salt should not need to be buffered (as the freeze-dried sodium salt has to be) to obtain stability. It is also expected that the amorphous form will have more rapid dissolution properties, which would be useful in certain dosage forms.

16. In conclusion, when compared to other available derivatives of ceftiofur, the hydrochloride salt represents a surprisingly and unexpectedly useful material. Both the crystalline and non-crystalline forms of ceftiofur hydrochloride salt have several advantages over other derivatives of ceftiofur and both have usefulness not only in chemical manufacture and purification, but also in pharmaceutical manufacture of useful dosage forms.

Date: 16Feb89

Jay D. Campbell

Attachments:
- Laboratory notebook pages 18774-JAC1-45,47;
- Laboratory notebook page 19706-ICPS-114.

ATTEMPT TO MAKE CRYSTALLINE HCl SALT OF THIOPHENE

SJL85 + HCl → ⊥ · HCl

17.3 N

mw	539.7		576.2
wt	.200		
mm	.37	3.96	
ρ	-		
vol	~	.11 ?.09	
source	17205-CES-117		

put thiophene in 25 ml erlenmeyer w/ stir bar
add: 3 ml acetone (TF)
1.5 ml H₂O (D.I.)
} ⇒ soln except for small glob

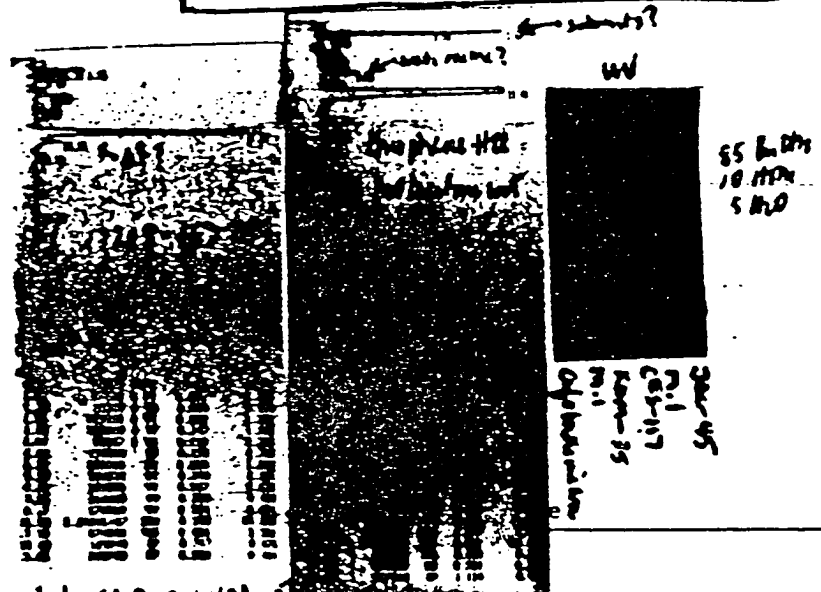
.1 ml HCl — all in soln, ⊥ yellow, RT

11:08 — begin heating with hot plate
1 ml H₂O — added improve stirring of final volume

11:15 T = 61°C
11:16 T = 67°C
11:18 T = 70°C — stop heat
11:19 .09 ml HCl added
T still went up to ~74°C → soln looks cloudy stir

12:29 60°C
cool, filter
13:15 2 x 1 ml 10% acetone in H₂O rinses
2 ml H₂O rinse
, vacuum dry @ 60°C O.N

SJL85 — JAL-45 149 mg 79.5%



very small crystals by microscopy

Date

HCl salt of cefotaxime see 1885-KOM-85

JUL 85

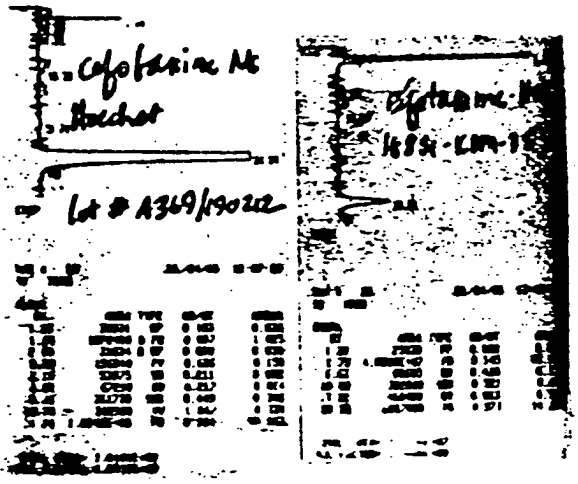
Background: KOM made HCl salt from Na salt using proc. very similar to JAC-45
- slow to crystallize
- cake "disappeared" (dissolved on washing w/ H2O)
- HPLC of solids was low in cefotaxime (decomposed?) ~50% yield
- solids looked crystalline
- heavy ppt in M.L.

crystals in M.L. were large - filtered nicely - large cake ~4g?
2x 4 mL 10% acetone in H2O rinses
2x 10 mL H2O rinse - shrinking of cake (dissolution? yes m.l. dried to solids)

cake went from granular looking to mud!
dry O.V. @ 60°C scrivelled up on drying

JAC-47 .67g

see TLC p. 45



sample given to Mike Dunn impurity was free as lactone

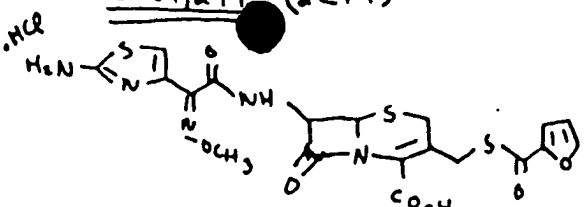
Read and understood by me

Date

K. Paul Shepherd
Jan. 12, 13 1988

Notebook # 19706

U-64,279A (2CFT)



m.w. 559.73

polyvinyl-pyridine → U-64,279 —HCl→ U-64,279A

a slurry of 20g. (0.03573 mole) of 2CFT (wt 2CFT 7002) and 6g. of polyvinylpyridine was stirred at room temp. for 1 hour with 200 ml. of 7% aq. acetone (14 ml. H_2O - 186 ml acetone). The slurry was filtered. The solids were washed with 3 x 30 ml. of 3% aq. acetone. The filtrate was diluted to a volume of 300 ml.

This filtrate was divided into 5 - 60 ml. portions.

Each 60 ml. portion contains $\frac{0.03573}{5} = 0.0071463$ mole of U-64,279.

conc aq. HCl
12N
37% by wt.

#1 A 60 ml. portion of the above solution was treated with 0.596 ml. of conc. aq. HCl (0.0071463 mole HCl)(1eq.) and evaporated to dryness. The solids were pulled on high vac. for two days to give 3.668g. of 2CFT.

19706-KPS-114-1

#2 A 60 ml. portion of the above solution was treated with 0.536 ml of conc. aq. HCl (0.006431 mole)(0.9 eq. of HCl) and evaporated to dryness. The solids were pulled on high vac. for two days to give 3.593g. of 2CFT.

19706-KPS-114-2

#3 A 60 ml. portion of the above solution was treated with 0.477 ml. of conc. aq. HCl (0.005717 mole; 0.8 eq. of HCl) and evaporated to dryness. The solids were pulled on high vac. for two days to give 3.562g. of 2CFT. * Product not crystalline

19706-KPS-114-3

#4 A 60 ml. portion of the above solution was treated with 0.417 ml. of conc. aq. HCl (0.005002 mole; 0.7 eq. of HCl) and evaporated to dryness. The solids were pulled on high vac. for two days to give 3.513g. of 2-CFT.

The 2CFT from #1, #2, and #4 all crystallized out during concentration and were isolated as crystalline solids. The 2CFT from #3 did not crystallize during concentration and was isolated as a non-crystalline foam.

Read and understood by me

Date